

# Performance of a Proposed Determinative Method for p-TSA in Rainbow Trout Fillet Tissue and Bridging the Proposed Method for Total Chloramine-T Residues in Rainbow Trout Fillet Tissue

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#### Introduction

Bacterial gill disease(BGD) is a predominant disease of fish cultured in crowded rearing conditions and is responsible for substantial production losses on federal, state, and commercial hatcheries. Chloramine-T (chl-T) is a disinfectant that is effective in treating BGD. Legal use of chl-T as a therapeutic drug in fish culture depends on approval by the Food and Drug Administration (FDA). Data required for an approval include depletion of the chl-T marker residue (para-toluenesulfonamide, p-TSA) from edible fillet tissue of exposed fish. Before conducting a depletion study, a method for determining p-TSA in fillet tissue had to fulfill FDA accuracy and precision criteria and show acceptable performance when compared to a former method for determining chl-T residues in fish fillet tissue.

# **Objectives**

- Evaluate the method accuracy and precision with p-TSA fortified rainbow trout fillet tissue.
- Evaluate the method precision with rainbow trout fillet tissue containing incurred p-TSA.
- 3) Bridge the current extraction method with a former chl-T residue method by mimicking the exposure of rainbow trout in the original chl-T total residue depletion study and comparing p-TSA concentrations in fillet tissue of fish exposed in this study with data from the original depletion study.



## **Extraction Method**

Homogenize fillet tissue with dry ice

Dry tissue with sodium sulfate

Extract p-TSA from tissue with methylene chloride

Pass extract through a silica gel solid phase extraction column

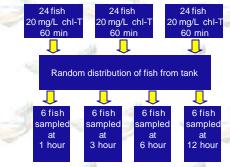
Elute p-TSA from column with acetonitrile and water solution

Analyze for p-TSA by HPLC





# **Chl-T Exposure for Method Bridging**



### **Objective 1 Results**

Method accuracy and precision with fortified fillet tissue.

Nominal p-TSA concentration	_	Mean%	% RSD
(ng/g) 500	n 7	92.6	% RSD 4.5
1000	7	93.4	2.5
2000	7	94.6	1.4

#### Objective 1 Conclusion

Method is accurate and precise with edible rainbow trout fillet tissue fortified with p-TSA at 0.5X , 1X, and 2X the expected 1000 ng/g tolerance limit.

# **Objective 2 Results**

Method precision with incurred p-TSA in fillet tissue.

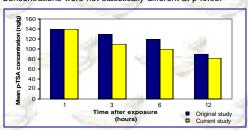
		Mean p-TSA	Method
	Fish	concentration	1
	precision		
	identification	(ng/g)	(% RSD)
ı	Fish 1	1020	2.4
	Fish 2	948	8.4
	Fish 3	1090	7.2
	Fish 4	1000	3.3
	Fish 5	1050	0.8
	Fish 6	1020	1.5

#### Objective 2 Conclusion

Method is precise with edible rainbow trout fillet tissue containing incurred p-TSA.

# **Objective 3 Results**

Incurred p-TSA concentrations in the fillet tissue from fish exposed in the original study and the current study. Concentrations were not statistically different at p<0.05.



#### **Objective 3 Conclusion**

The proposed method was successfully bridged to the former method used in the original depletion study. Concentration of p-TSA determined in the two studies were not statistically different.